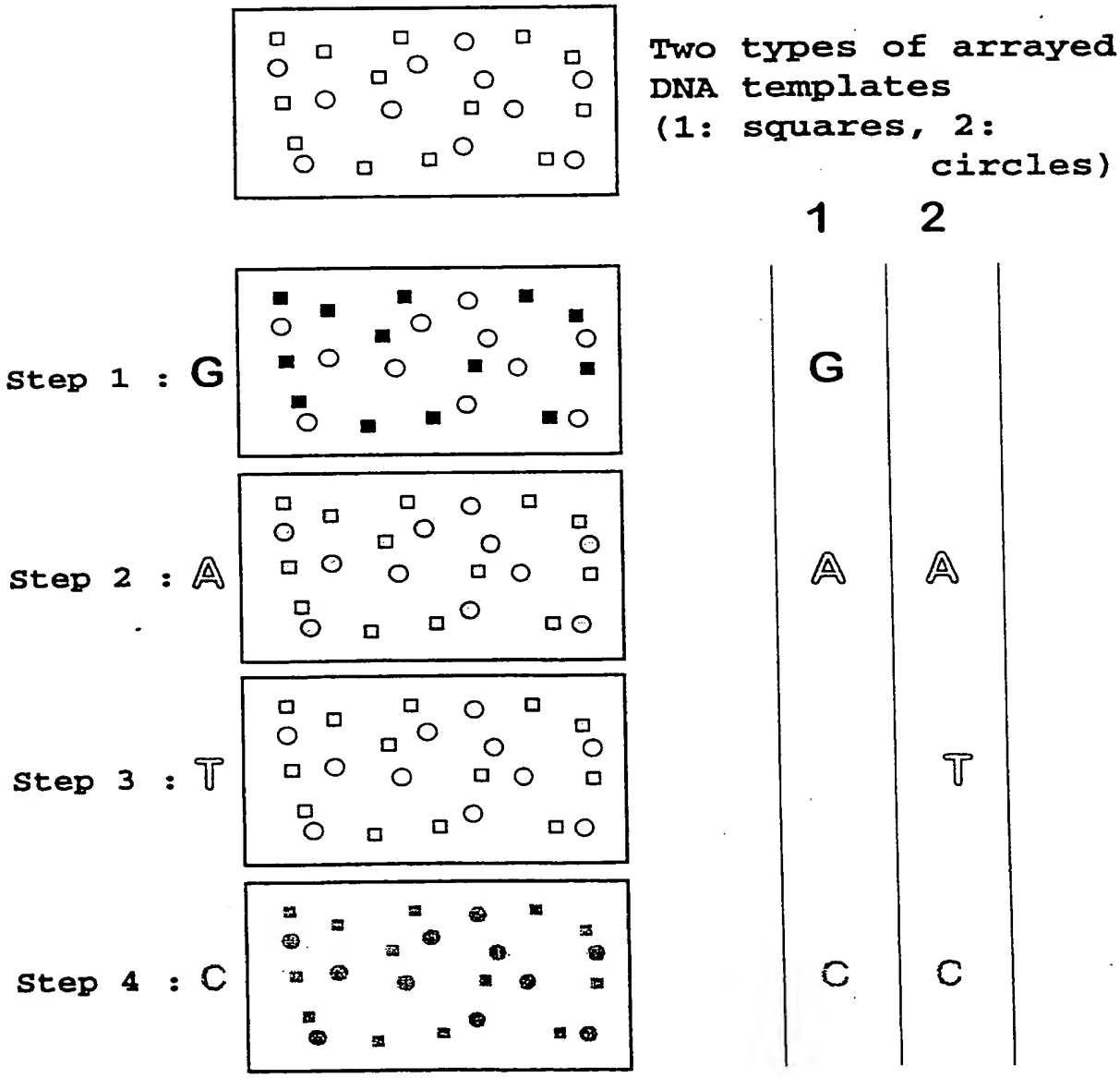
FIG. 1.

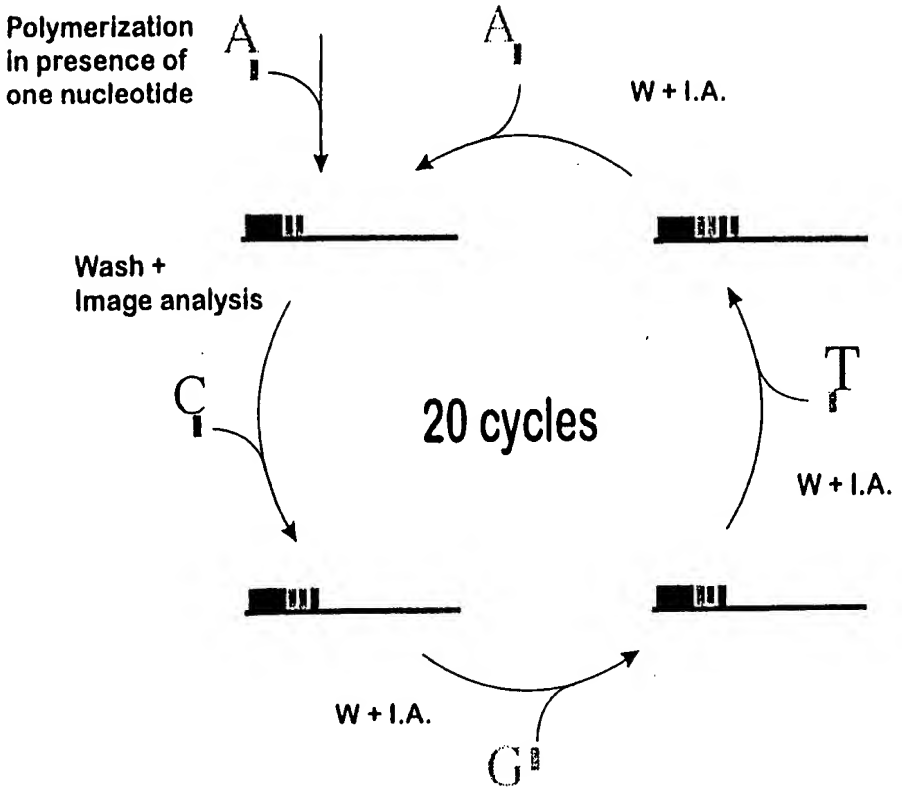

in situ Sequencing
Two types of arrayed DNA templates (1: squares, 2: circles)
1
2
Step 1 : G
Step 2 : A
Step 3 : T
Step 4 : C
G
A
A
T
C
C
Sequence from type 1 : G A C
type 2 : A T C

in situ Sequencing

FIG. 2.

```
Primer :          5'-gactagcgtcat-3'
                     ||||||||||||
Template :  3'-ggatgctgatcgcactattgatgggcacgaactca-5'
```

Cycle of stepwise base extension



Fluorescence increase on individual spots

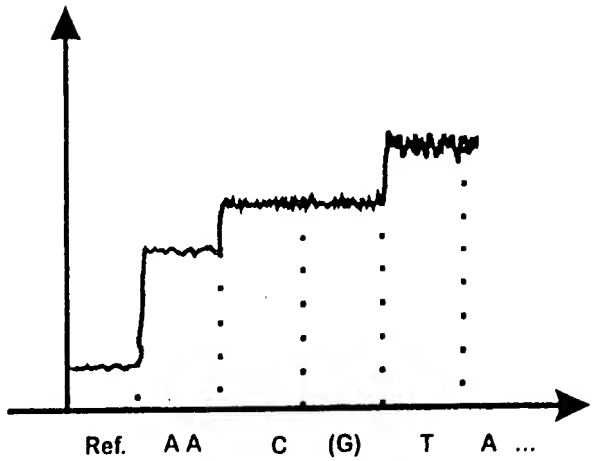


FIG. 3.

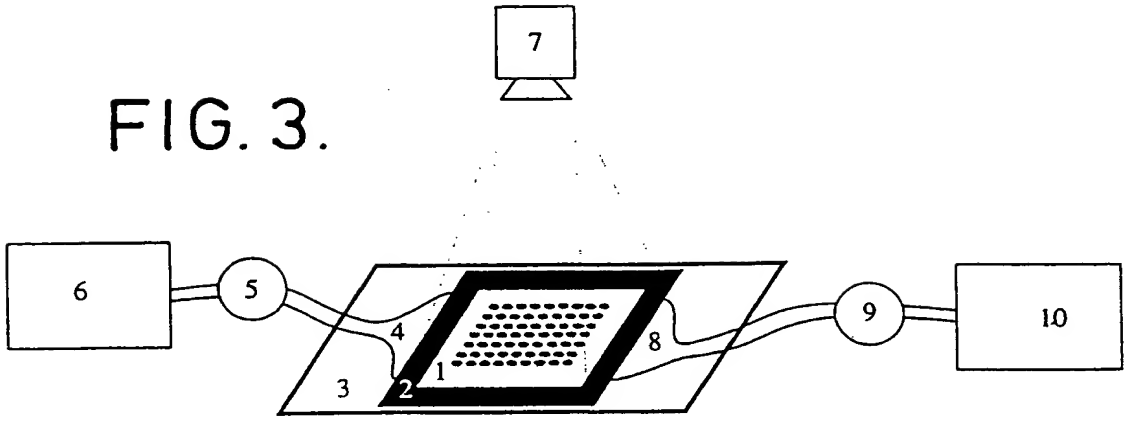


FIG. 4.

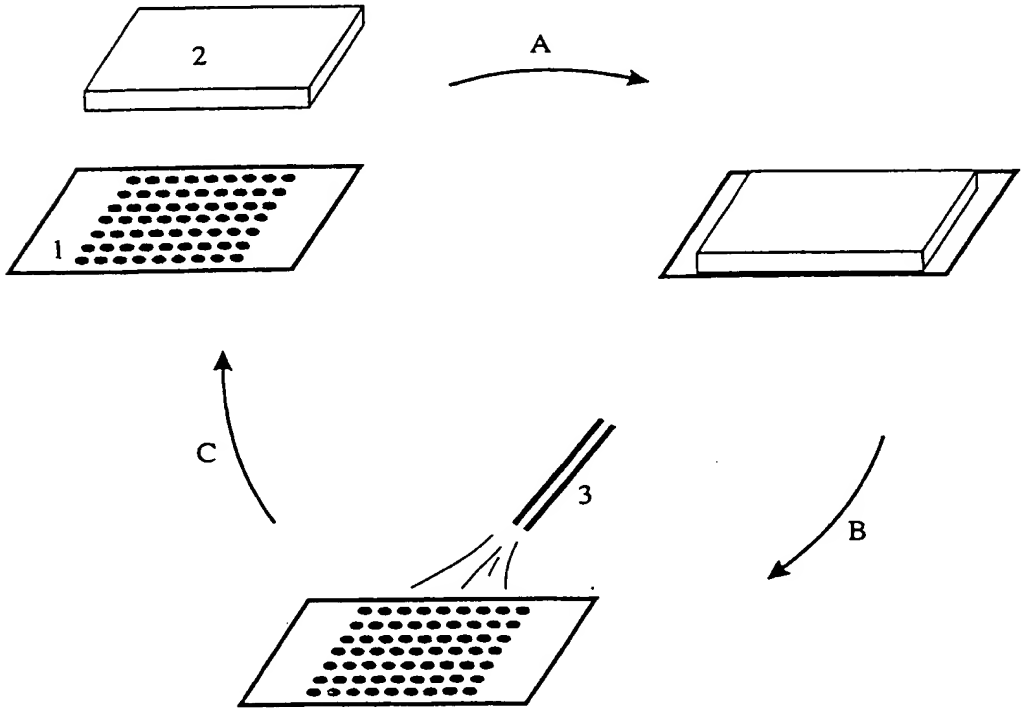
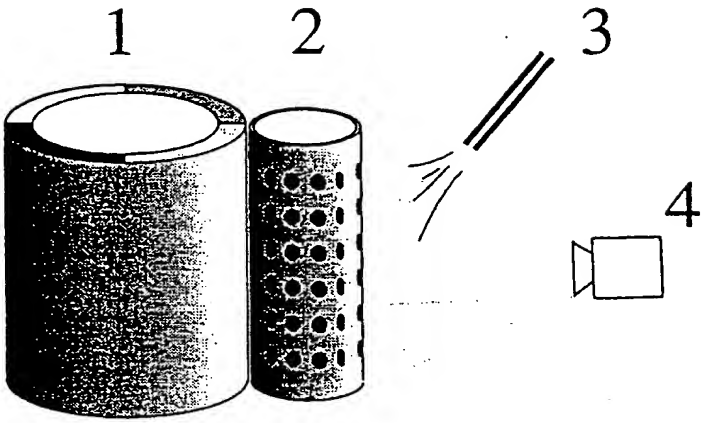


FIG. 5.

A.



B.

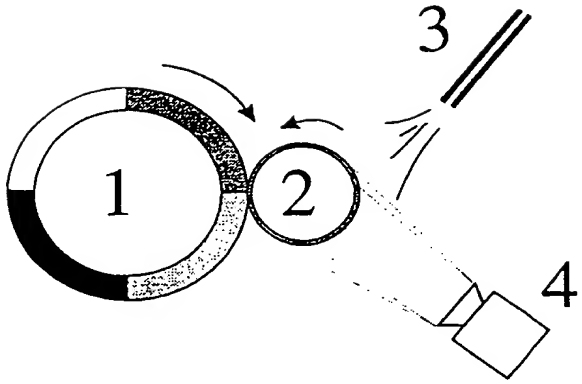
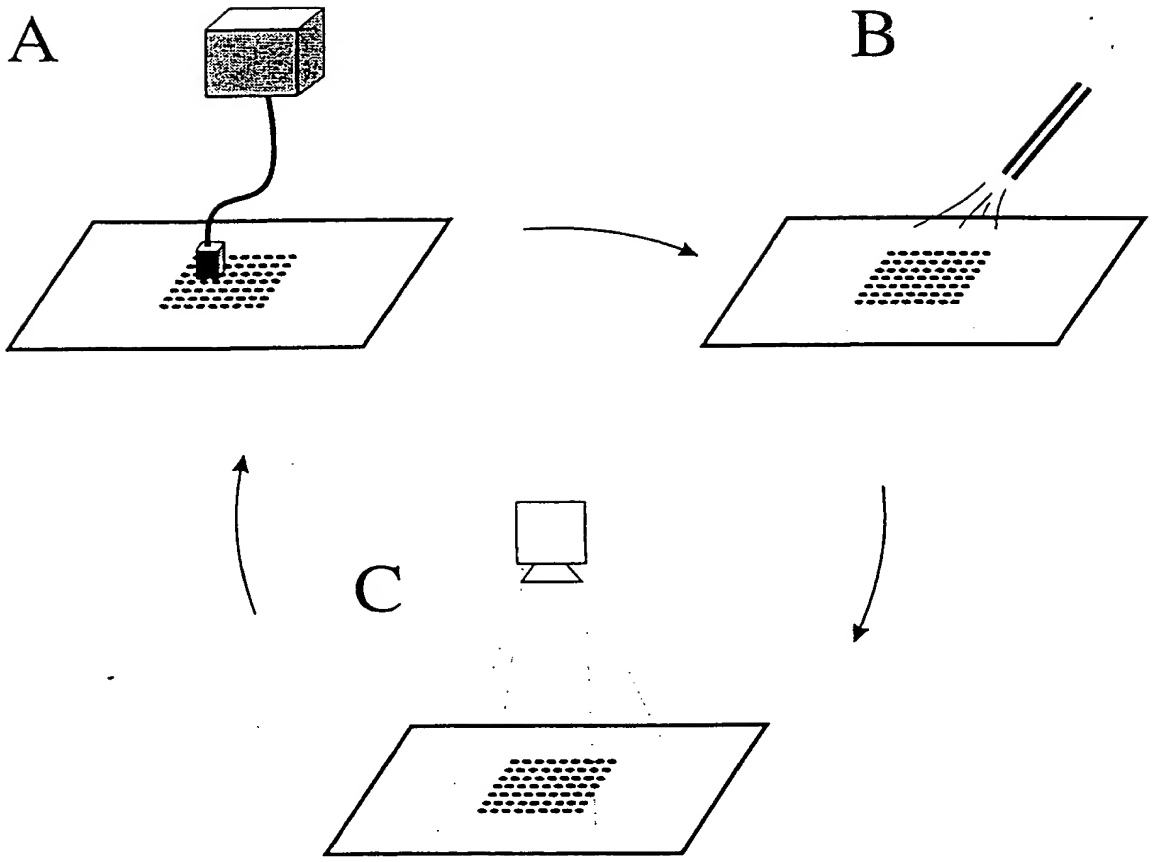


FIG. 6.



in situ Sequencing : *de novo* Sequencing

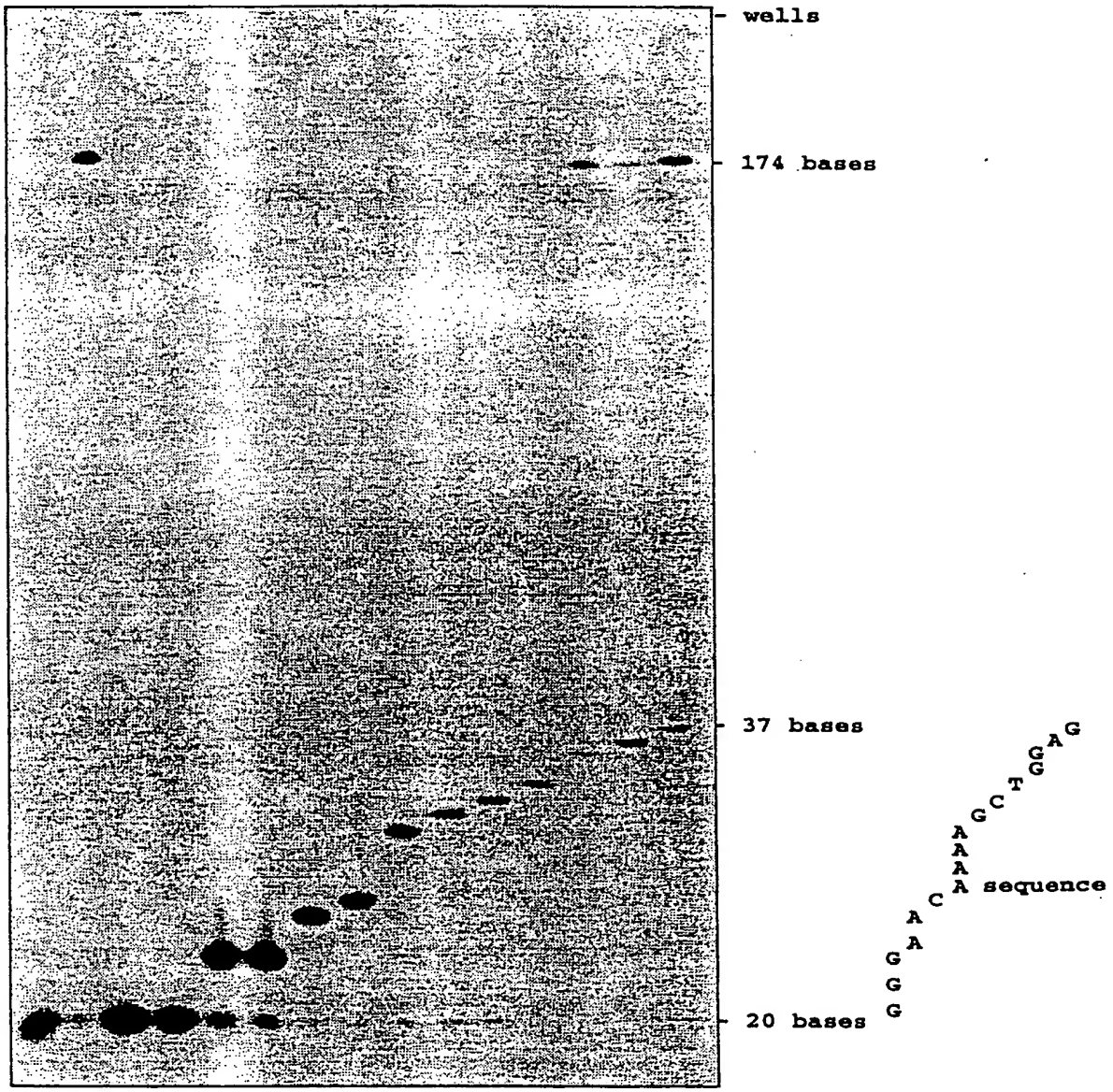


FIG. 7.

FIG.8.

in situ Sequencing : Non-labelled primers

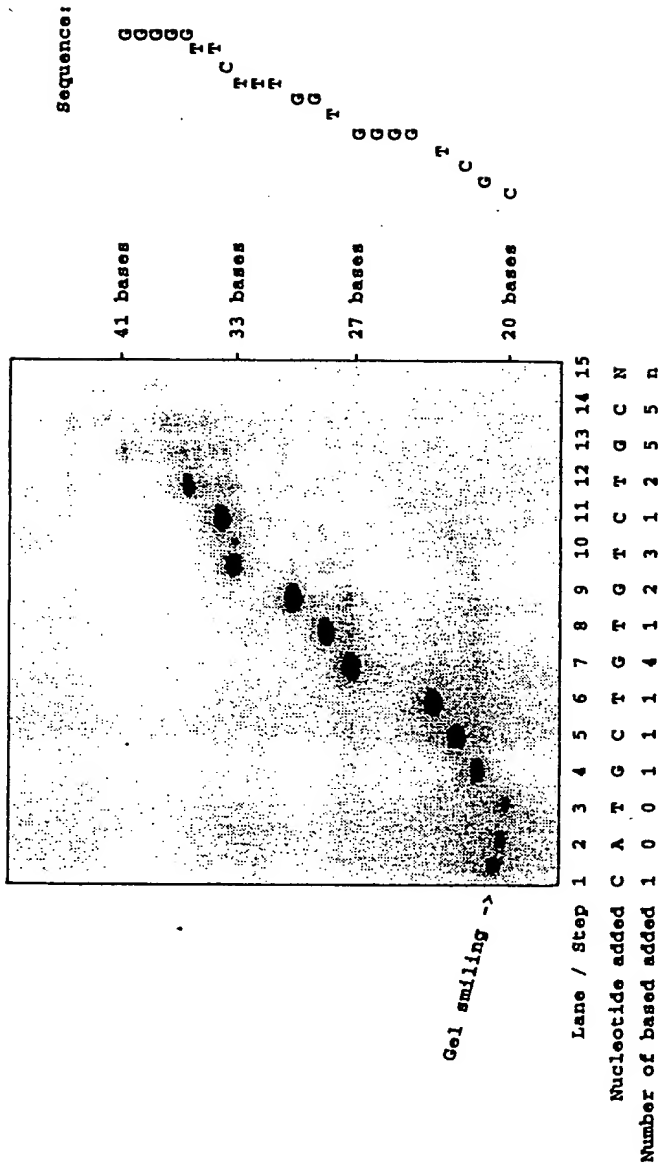


FIG. 9.

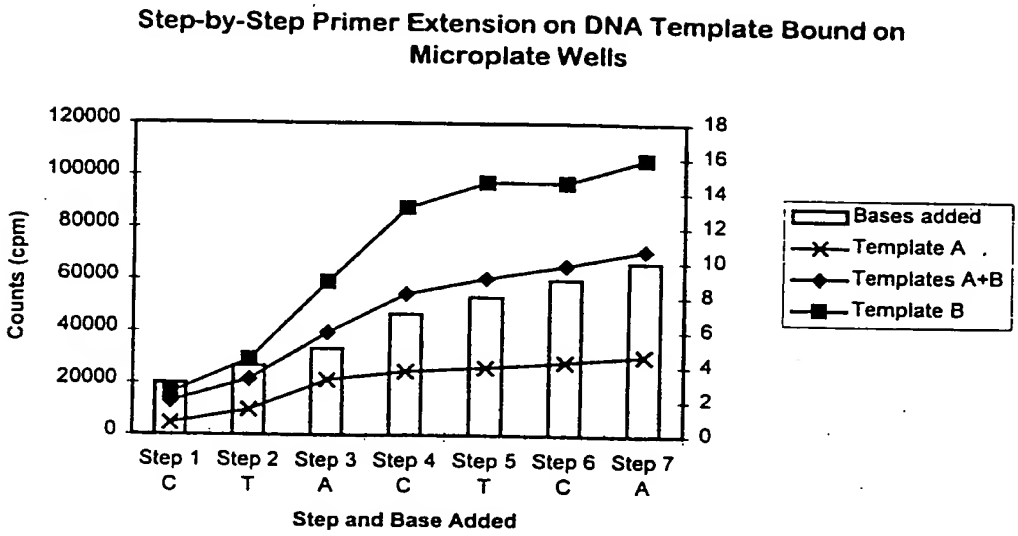

Step-by-Step Primer Extension on DNA Template Bound on Microplate Wells
Counts (cpm)
120000
100000
80000
60000
40000
20000
0
18
16
14
12
10
8
6
4
2
0
Step 1 C
Step 2 T
Step 3 A
Step 4 C
Step 5 T
Step 6 C
Step 7 A
Step and Base Added
Bases added
Template A
Templates A+B
Template B

FIG. 10.

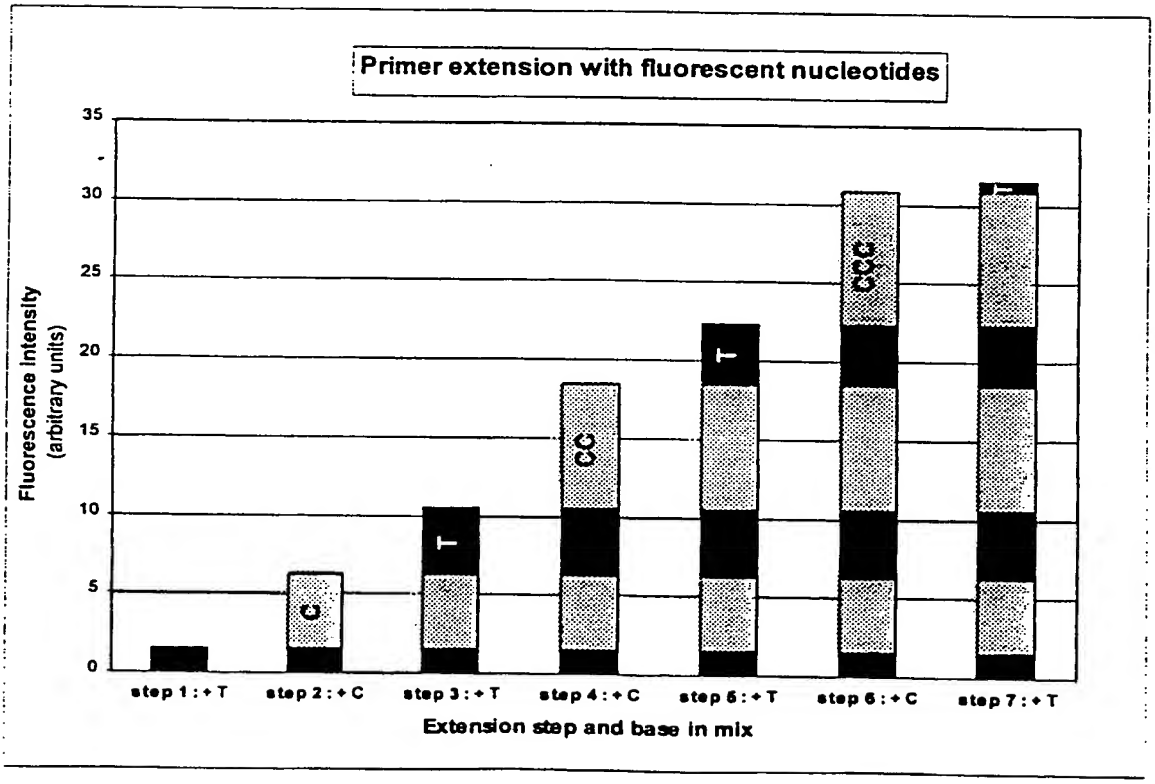

Primer extension with fluorescent nucleotides
Fluorescence Intensity (arbitrary units)
35
30
25
20
15
10
5
0
C
T
CC
T
CCC
T
step 1 : + T
step 2 : + C
step 3 : + T
step 4 : + C
step 5 : + T
step 6 : + C
step 7 : + T
Extension step and base in mix

FIG. 11.

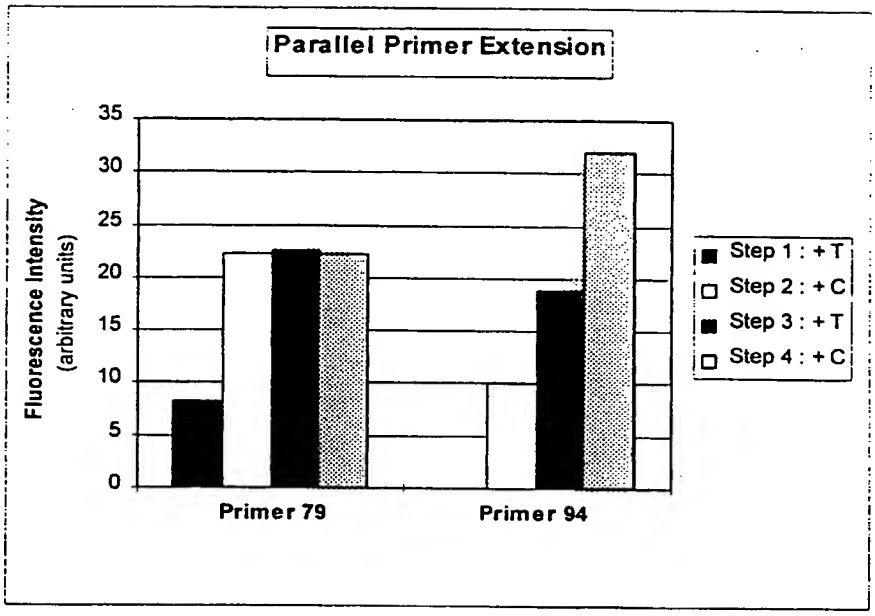


Table 11:

Step	Base in mix	Primer 79 : TCGA			Primer 94 : CTCA		
		Expected extension	Bases added	Fluorescence intensity	Expected extension	Bases added	Fluorescence intensity
1	T	T	1	8	none	0	0
2	C	C	2	22	C	1	10
3	T	none	2	23	T	2	19
4	C	none	2	22	C	3	32